Immunoperoxidase Staining of Fos Protein in the Male Rat Brain After Exposure to Female Pheromones

by

Linda Olson

Submitted in Partial Fulfillment of the Requirement
for the Degree of
Master of Science
in the
Biological Sciences Program

Robert E. Lipschutz — 6/3/94

Advisor — Date

Dean of the Graduate School — 6/6/94

Date

ORAL EXAMINATION APPROVAL FORM

Linda Olson has successfully defended a thesis

entitled Immunoperoxidase Staining of Fos Protein in the Male Rat Brain After Exposure to Female Pheromones

by oral examination on May 18, 1994.

| COMMITTEE MEMBERS: | AGREE | DISAGREE |
|---|---|---|
| Robert E. Leipheimer | ✓ | |
| Diana Fagan | ✓ | |
| Johanna K. Krontiris-Litowitz | ✓ | |

DATE 6/3/94

THESIS APPROVAL FORM

THESIS TITLE: Immunoperoxidase Staining of Fos Protein in the Male Rat Brain After Exposure to Female Pheromones

AUTHOR: Linda Olson

DEGREE: Master of Science

ADVISOR: Dr. Robert Leipheimer

| COMMITTEE MEMBERS: | ACCEPT | REJECT |
|---|---|---|
| Robert E. Leipheimer | ✓ | |
| Diana Faegan | ✓ | |
| Johanna K. Kreulen Utrecht | ✓ | |

DATE 6/3/94

ABSTRACT

Immunoperoxidase Staining of Fos Protein in the Male Rat Brain After Exposure to Female Pheromones

Linda Olson

Master of Science

Youngstown State University

Electrolytic lesion studies and neurotransmitter release studies suggest that sexual behavior in the male rat is regulated by specific brain areas such as the medial preoptic area (MPOA), olfactory bulbs, and the nucleus accumbens. Also, testosterone has long been established as a critical factor in the regulation of sexual behavior. This experiment focuses on the motivational aspects of sexual behavior in the male rat. The effects of exposure of intact and castrated male rats to bedding samples from estrous females were assessed in specific brain areas through immunoperoxidase staining for fos, the protein product of the proto-oncogene c-fos. C-fos has been shown to function in signal transduction in cells. Consequently, fos levels serve as an excellent indicator of neuronal activity. In these experiments, exposure to estrous bedding increased the number of fos stained nuclei in the MPOA, nucleus accumbens, diagonal band of broca(DBB), and corpus striatum of intact male rats. Castration blocked the

increase in fos in the MPOA, nucleus accumbens, and corpus striatum. Castration did not block the increase in the DBB. The olfactory bulbs showed equal amounts of staining in all groups. These results indicate that the MPOA, nucleus accumbens, and corpus striatum, are involved in processing sexually relevant cues from the environment and that testosterone is involved in regulating the process. In contrast, the increase in fos staining in the DBB in response to estrous bedding appears to be independent of testosterone.

## ACKNOWLEDGEMENTS

I would like to thank all of my family and friends for providing me moral support through my many years of school. For technical assisstance, I would like to thank Magdi Salim from Kent State University and a very special thank you to Dave Hamrock for all of his help. Most of all, I would like to thank Dr. Leipheimer for giving me the opportunity to work in his lab and for his unending enthusiasm for the work we accomplished. Finally, I would like to thank the members of my committee, Dr. Fagan and Dr. Litowitz, for donating their time.

TABLE OF CONTENTS

# LIST OF FIGURES

# CHAPTER I

## INTRODUCTION

In animals, physical activities including sexual behavior can be viewed as having two distinct components. The first aspect of a behavior is an anticipatory or motivational stage, which is followed by the consummatory stage that is characterized by increased motor activity (Mogenson et al., 1980). Structures in the forebrain such as the amygdala and hippocampus are thought to interpret data from the environment which establishes the motivational state of the animal. This information is then transferred to the nucleus accumbens for translation into the appropriate motor response (Mogenson et al., 1980). The activity of an animal can be initiated by changes in internal conditions which require homeostatic regulation or by changes in the external environment which may instigate action. The motivational state of sexual activity can be examined by exposure of male rats to female pheromones (Louilot et al., 1991). The bedding from estrous females contains urine, which is a source of pheromones (Vandenbergh, 1988). Exposure of male rats to female pheromones is an effective stimulus as Hlinak et al. (1984) reported that male rats exposed to urine from estrous females showed increased precopulatory activity.

If the males are not given the opportunity to copulate following exposure, the effects of consummatory behavior are eliminated. A study such as this focuses on the effects of anticipatory behavior uncomplicated by the vast effects of motor activity involved in copulation. Several brain areas are thought to be involved in interpreting sensory data from the environment, establishing the motivational state, and translating this information into consummatory action. Some of these brain areas are thought to be the medial preoptic area, nucleus accumbens, and the olfactory bulbs.

The regulation of copulatory behavior in the male rat by certain areas of the brain has been studied through various methods such as electrical stimulation, electrolytic lesions, the study of hormonal effects, and the effects of various pharmacologic agents. Much of the data that has been compiled points to the critical roles played by the olfactory bulbs, the medial preoptic area (MPOA), and possibly the nucleus accumbens in the mediation of sexual activity in the male rat. The olfactory bulbs are thought to participate in the establishment of the motivational state of the animal. While the MPOA and nucleus accumbens are thought possibly to participate in anticipatory or consummatory aspects of sexual behavior (Edwards and Einhorn, 1986). Figure 1 summarizes the neural pathways that exist between various areas of the rodent brain. As depicted in the figure, the olfactory bulbs convey messages to the amygdala and hippocampus in the forebrain. The amygdala and hippocampus have neural connections with the bed

nucleus of the stria terminalis and MPOA which relay messages to the ventral tegmantal area (VTA). The VTA then transfers the signal to the nucleus accumbens, where the signal is relayed to areas of the brain which are responsible for motor activity.

MEDIAL PREOPTIC AREA

Since the MPOA is located in the vicinity of the olfactory telencephalon, the midbrain tegmentum, and the gonadotrophic mechanisms of the hypothalamic region, a natural observation would be to assume that it may have a role in the regulation of sexual behavior (Heimer and Larsson, 1966/67). Lesion studies of the MPOA have been very useful in elucidating the pivotal role that this brain area assumes in the facilitation of copulatory behavior in the male rat. Heimer and Larsson (1966/67) found that large lesions of the MPOA that extend to the rostral anterior hypothalamus eliminated copulatory behavior in male rats and attempts to reactivate behavior by handling the subjects, replacing females, or testosterone therapy were ineffective in restoring behavior. The lesioned males in their study did not copulate but some displayed genital sniffing and pursuit of the female. Sachs and Meisel (1988) state that disruption of copulation in MPOA lesioned males may not be totally a result of reduced sexual motivation but may be due to a failure to couple sensory information with the proper behavioral response. In contrast, Chen and Bliss (1974) performed bilateral or unilateral preoptic lesions along with bilateral or unilateral lesions of the mammillary bodies in sexually experienced male rats.

They reported that mammillary lesions had no effect on sexual behavior, whereas complete destruction of the MPOA completely abolished mating behavior and incomplete ablation increased mount, intromission, and ejaculation latencies. Since they found no change in the post ejaculatory-refractory interval in those animals able to copulate, they concluded that the MPOA is involved in a sexual arousal mechanism.

The problem that arose in lesion studies of the MPOA was that the size and location of the lesion had variable effects on the severity of the deficit in behavioral output. It was noted that smaller lesions usually resulted in slight deficits of copulatory behavior or full recovery of behavior. Heimer and Larsson (1966/67) compared the effects of large and small lesions of the medial preoptic-anterior hypothalamic continuum. The large lesions of the MPOA abolished mounting, intromission, and ejaculation. Smaller lesions along the continuum did not abolish copulatory behavior and the temporary deficits were not caused by ablation of any specific part of the continuum. They concluded from this that the neurons which are responsible for mating behavior are spread out along the medial preoptic-anterior hypothalamic continuum. Evidence contradictory to this assumption was presented by van de Poll and van Dis (1977) who studied bisexual behavior in male rats with medial preoptic-anterior hypothalamic lesions. They reported that bilateral lesions at the transition of the MPOA and anterior hypothalamus caused a large deficit in masculine sexual activity, while lesions centered in the anterior hypothalamus facilitated

feminine behavior in male rats. They concluded that the medial preoptic-anterior hypothalamic continuum is divided into distinct functional and anatomic units.

In addition to the essential connection between the MPOA and the anterior hypothalamus, there is evidence that the efferent neurons from the MPOA that pass through the medial forebrain bundle and terminate in the midbrain are also crucial in sexual behavior of the male rat. Brackett and Edwards (1984) demonstrated that bilateral lesions of the dorsolateral tegmentum (DLT) eliminated sexual behavior and postulated that the MPOA and DLT are part of a system that integrates forebrain and midbrain control of sexual behavior. To test this theory, they coupled a unilateral preoptic lesion with a contralateral DLT lesion and noted an elimination of sexual behavior. This supports the theory that connections between these two areas are essential for sexual behavior. Edwards and Einhorn (1986) performed MPOA and DLT lesions which abolished copulation and decreased preference for a receptive female. The lesioned rats were later castrated and given testosterone therapy which was ineffective in restoring preference for a receptive female to the level of castrated control rats given testosterone. They concluded that castration and brain lesion decrease sexual motivation and that sex hormones may act on the MPOA to increase sexual motivation.

Since electrolytic lesions of the MPOA have been shown to be detrimental to male rat sexual activity, it would seem logical that electrical stimulation of this particular brain area would enhance

sexual activity. Several investigators have used electrical stimulation of the MPOA to show a facilitation of male sexual behavior in the rat. van Dis and Larsson (1971) reported dramatic increases in copulatory activity in some male rats following electrical stimulation of the MPOA. Malsbury (1971) compared the effects of MPOA and lateral preoptic stimulation. He reported a reduction in the number of mounts and intromissions preceding ejaculation in rats with MPOA stimulation but not in rats with lateral preoptic simulation.

The importance of endocrine regulation of sexual behavior has been known for many years, as castration used for other purposes resulted in a reduction of sexual behavior. The ability to display sexual behavior is dependent on the presence or previous exposure to gonadal hormones, however, some questions still remain about the site of action of the hormones. They either act on peripheral organs or on specific brain areas that organize the behavior. Davidson (1966) reported that restoration of sexual behavior in castrated male rats can be achieved by exposure of brain tissue to testosterone and that testosterone can act directly on the brain. Implantation of crystalline testosterone propionate into the hypothalamic-preoptic area of castrated male rats was effective in restoring sexual behavior, and implantation into the MPOA was found to cause the most consistent change. This implies that gonadal hormones can exert their actions at the level of the brain, especially hypothalamic-preoptic regions, and that sexual behavior may be independent of peripheral mechanisms (Davidson, 1966).

Further support for this idea was obtained by Sar and Stumpf (1973) who injected castrated male rats with tritiated testosterone and examined brain sections for radioactivity. They reported that the highest areas of labeling were in the arcuate nucleus, ventromedial hypothalamus, MPOA, stria terminalis, and amygdala, which are all areas that are implicated in the regulation of gonadotropin release and male sexual behavior.

NUCLEUS ACCUMBENS

The nucleus accumbens is the area of the brain which is thought to bridge the gap between the limbic system, which is responsible for assessing the environment of the animal and determining its motivational state, and areas of the brain that are responsible for translating these impulses into actions (Mogenson et al., 1980). Anatomical data and behavioral evidence demonstrate that the nucleus accumbens is innervated by dopaminergic neurons that have a locus in the ventral tegmental area, which in turn has neural connections with limbic forebrain structures such as the medial amygdala and hippocampus (Mogenson et al., 1980). The nucleus accumbens has also been shown through various experiments to have GABAergic neural connections to the globus pallidus which is part of the basal ganglia motor system (Mogenson et al., 1980). Neural messages from the limbic system which contain information about the motivational state of the animal are conveyed to the nucleus accumbens and then transmitted to the globus pallidus which allows the animal to act on its motivations.

Initial experiments investigating the effects of dopamine were accomplished by injection of the synthetic dopamine precursor L-dopa, and recording subsequent changes in behavior of the animal. Tagliamonte et al. (1974) reported a marked increase in mounts and intromissions of sluggish male rats after injection with L-dopa and the decarboxylase inhibitor RO4-4602 (RO4-4602 increases the amount of dopamine reaching the brain by preventing the decarboxylation of L-dopa in peripheral tissues). Since it was determined that L-dopa is also a precursor of other catecholamines and not specifically dopamine, subsequent studies utilized dopamine receptor agonists such as apomorphine and antagonists such as haloperidol or lisuride to assess the role of dopamine in the brain. Paglietti et al. (1978) showed a decrease in the number of intromissions to ejaculation and accelerated ejaculation in sexually experienced male rats treated with apomorphine. Pharmacologic agents such as these interfere with unintended receptors and also have dose-dependent differences in action. Malmnas (1976) for example, reported that lower doses of L-dopa facilitated sexual behavior while higher doses of L-dopa inhibited sexual behavior in castrated male rats also treated with testosterone.

Results obtained from the use of pharmacologic agents are useful but must be interpreted with caution due to dose-dependent responses and the interaction of drugs with unintended receptors. Other methods have been developed to actually measure levels of dopamine in the brain. Two methods that are currently used to measure the amount of extracellular dopamine in the brain of the

freely moving rat are microdialysis and *in vivo* voltammetry. Microdialysis involves inserting a probe with attached tubing into the selected brain area and analyzing the dialysate by high pressure liquid chromatography. *In vivo* voltammetry is accomplished by inserting a carbon fiber micro-electrode into the desired brain area and compiling differential normal pulse voltammograms for the desired compound. The advantage of these procedures is that they specifically measure levels of dopamine in a determined brain area with less interference from other compounds. Microdialysis produces highly specific results due to the use of high pressure liquid chromatography, while time resolution is sacrificed. Voltammetry produces data with lower specificity but allows greater time resolution.

The involvement of the nucleus accumbens in motor activity is thought to be a result of a dopaminergic mechanism. Injection of pharmacologic agents which enhance dopamine transmission into the nucleus accumbens have been shown to increase motor activity. McCullough and Salamone (1992) used a microdialysis procedure to demonstrate that periodic food presentation, which has been shown to instigate considerable motor activity in food deprived rats, does so through an increase in dopamine release in the nucleus accumbens. Louilat et al. (1986) measured the reactivity of dopaminergic neurons using *in vivo* voltammetry as related to different social interactions. They observed a large increase in dopamine when rats were placed with an aggressive male cogener. They concluded that environmental stimuli are responsible for

activation of the neurons and that the amount of stimulation depends on characteristics of the stimulus. West et al. (1992) performed an interesting experiment in which they trained male rats to associate certain odors with a receptive female to determine if this could change later responses of nucleus accumbens neurons. In trained males, they reported an increase in neuronal activity in response to odors associated with a receptive female. The authors concluded that associating odors with receptive females caused an increased responsiveness of nucleus accumbens neurons to these odors, supporting a role for the nucleus accumbens in pairing stimuli from the environment with reward processes.

Since the nucleus accumbens has been associated with motivated behaviors and sexual behavior can be viewed as a motivated behavior, many studies have been performed to determine how sexual behavior is influenced by the nucleus accumbens. Some investigators have focused on copulation while others have concentrated on stimuli which accompany sexual behavior in the male rat. Accumbal dopamine levels of castrated male rats are decreased. Testosterone prevents this decrease, implying that testosterone affects the mesolimbic dopamine system, which is involved in mediating sexual arousal (Mitchell and Stewart, 1988). Louilot et al. (1991) performed a voltammetric study to determine dopamine concentrations in the nucleus accumbens of inexperienced male rats which were exposed to olfactory stimuli from a receptive female, a non-receptive female, and an intact male. They found a significant increase in dopamine levels only in those rats exposed to odors

from the receptive female and concluded that the nucleus accumbens is involved in the recognition of relevant olfactory cues. Dopamine levels in the nucleus accumbens also have been shown to increase when experienced male rats are placed in mating chambers with a receptive female behind a screen partition (Pfaus et al., 1990). Mitchell and Gratton (1991) found an increase in accumbal dopamine when male rats were exposed to bedding of estradiol-progesterone primed female rats as opposed to bedding from ovariectomized females or male rats. These data support the role of the nucleus accumbens in processing cues obtained form the environment of the animal.

Other investigators have focused on the measurement of dopamine release in the nucleus accumbens during copulation. Pleim et al. (1990) used microdialysis to compare extracellular levels of dopamine and 3,4 dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens of male rats during copulation versus mild tail pinch. They reported significant increases only during copulation, thus implying that rewarding stimuli are connected with dopamine release in the nucleus accumbens. Mas et al. (1990) reported increased dopamine levels in the nucleus accumbens that coincided with the start of mounting activity, which supports a role for the nucleus accumbens in the transfer of environmental stimuli to the motor system. Damsma et al. (1992) reported that copulation increases dopamine levels in the nucleus accumbens and striatum of male rats. Accumbal dopamine is also increased during the presentation of a female behind a screen but striatal dopamine is not increased. This

data demonstrates that dopamine transmission in the corpus striatum may mediate the motor aspects of copulation. Thus, results of several experiments have provided correlative evidence supporting a role for dopamine in the nucleus accumbens in modulating the neural mechanisms that regulate copulatory behavior in male rats.

OLFACTORY BULBS

The olfactory bulbs in the rat consist of two distinct components, the main olfactory bulb, which receives innervation from receptors in the nasal epithelium, and the accessory bulb, which is innervated by the vomeronasal organ. The most common method of studying disruption of the main olfactory bulb is through destruction of the nasal receptors by application of zinc sulfate. In order to study the functions of the accessory bulbs, the vomeronasal organ is removed or the vomeronasal nerve is cut. The olfactory bulbs of male rats are known to have at least some effect on copulatory behavior. Initially, studies into the effects of the olfactory bulbs on sexual behavior were aimed at the loss of what was thought to be a purely sensory organ. Much data was compiled in this area and eventually theories arose implying that the olfactory bulbs are in fact an integral part of the limbic system. Cain (1974) suggested that the olfactory bulbs modulate the excitability of forebrain regions since olfactory bulbectomy abolishes interspecific aggression in rats. deGroot (1991) states that in early neuroanatomical studies, the limbic lobe was considered to be involved in olfaction and was part of the rhinencephalon. Later,

functions of the limbic system were expanded to include feeding behavior, aggression, and emotional expressions, as well as the autonomic, behavioral, and endocrine aspects of the sexual response. All of these behaviors may be triggered by smell.

Early experiments by Heimer and Larsson (1967) in which the olfactory bulbs of sexually experienced male rats were lesioned resulted in prolonged latency periods, a reduced number of ejaculations, and increased the tendency for total unresponsiveness toward the female. Further experimentation investigated the role of previous sexual experience in copulatory behavior of bulbectomized rats. It was suggested that the olfactory bulbs are more important in the initiation of sexual behavior than in maintenance of the behavior in male rats (Larsson, 1975). Larsson (1975) performed bulbectomies on sexually inexperienced male rats prepubertally and postpubertally and found that the occurrence of sexual behavior was eliminated in most experimental animals. Bermant and Taylor (1969) performed unilateral and bilateral bulbectomies on experienced and inexperienced male rats and also tested the Coolidge Effect, the enhancement of the copulatory rate by introduction of a novel female. The experienced males with bilateral bulbectomies had prolonged ejaculation latencies in the first two series with one female. These deficiencies were overcome by the introduction of a novel female and unilaterally bulbectomized animals showed no deficit. The bilaterally bulbectomized virgin males had infrequent ejaculations but intromissions were not effected. The copulatory

deficits reported by these investigators were interpreted as being due to the resultant anosmia.

One possible explanation for the copulatory deficits seen in bulbectomized male rats was thought to lie in gonadal or gonadotrophic hormone failure, a possibility that was later eliminated. Larsson (1969) reported that in bulbectomized male rats that showed a sexual deficit, the behavior was not restored by injection of long lasting testosterone, FSH, or LH. These results disputed the theory that copulatory deficits in anosmic rats were due to hormonal failure.

The role of the olfactory bulb as a purely sensory organ has been disputed and some have proposed that in addition to the sensory role, the olfactory bulb has a role in modulation of the limbic system (Cain, 1974). One reason for the expansion of the role of the olfactory bulb from a purely sensory role is its involvement in activities that aren't apparently related to olfaction. Lumia et al. (1992) noted a significant decrease in the ratio of nocturnal wheel running in bulbectomized rats. Further support for the theory of involvement of the olfactory system in a central arousal mechanism was gained through studies investigating the effects of tail pinch and flank shock on bulbectomized rats. Wang and Hull (1980) performed tail pinch procedures on non-ejaculating bulbectomized male rats prior to testing with a receptive female. They found that tail pinch applied before copulatory testing can induce copulatory behavior in non-ejaculating bulbectomized male rats. The effects of flank shock

were similarly studied by Meisel et al.(1980). They performed unilateral and bilateral bulbectomies on experienced male rats and found severe copulatory deficits in bilaterally bulbectomized rats. Flank shock was found to improve their performance although they still had longer intromission and post-ejaculatory intervals than controls. Of interest, was their finding that the bilaterally bulbectomized rats that ejaculated with flank shock ejaculated a second time without shock. When these animals were tested the next week without shock, they failed to copulate. These authors cite that similar behavior results from medial forebrain bundle lesions but that this behavior is not restored by flank shock. This is due to disruption of the nigrostriatal dopamine system. They theorized that in bulbectomized rats, the dopamine system is not disrupted and that copulatory deficits are due to noradrenergic depletion.

Evidence has also been presented with regard to a possible serotonergic pathway in which the olfactory system is involved. Lumia et al. (1992) postulated that olfactory bulbectomized rats are a model for hyposerotonergic depression. Antidepressant drugs were used to increase copulatory frequency in bulbectomized rats and also to increase nocturnal activity in these animals. They also measured serotonin(5-HT), dopamine, norepinephrine and their metabolites in frontal cortex, striatum, hippocampus, and nucleus accumbens of bulbectomized rats. They reported an increase in 5-HT of 30% in frontal cortex, hippocampus, and striatum, while the level in the nucleus accumbens was unchanged. Dopamine and norepinephrine were apparently not affected by bulbectomy.

The olfactory cortex is connected to a brain area known as the diagonal band of broca (DBB) (Luskin and Price, 1982). The DBB shares reciprocal connections to areas of the limbic system such as the dorsal and ventral regions of the hippocampus (Hjorth-Simonsen, 1971). The hippocampus is an area which is thought to be involved in learning (deGroot, 1991). Roman et al. (1993) tested the hypothesis that the DBB is a critical link between the limbic system and the olfactory cortex with regard to learning and storage of olfactory information. They reported that DBB lesions in rats impaired odor-reward association when intervals between trials were greater than 15 seconds. This implies that the DBB can function in the memory storage of olfactory cues.

Brain lesion and electrical stimulation, as well as measurement of various chemicals in the brain have produced much information regarding the importance of specific brain areas in the regulation of sexual behavior in male rats. Another method currently used is immunoperoxidase staining of the protein fos. Fos is the protein product of the proto oncogene c-fos, and can be used as a marker of neuronal activity. Quantitation of the number of stained nuclei can be used to determine the activity of specific brain regions.

C FOS

An oncogene is a gene that is capable of inducing a malignant phenotype through its abnormal expression or altered protein products. The cellular origin of oncogenes was established when a

radioactive probe for the src viral oncogene was developed and normal mammalian cells were reported to contain these sequences (Cooper, 1990). The cellular sequences in normal cells from which viral oncogenes are derived are called proto-oncogenes.

Proto-oncogenes have normal physiological and biological activity in cells. The main function of proto-oncogenes in the normal cell seems to be in the process of signal transduction, the transfer of information between and within cells (Morgan and Curran, 1991). The proto-oncogenes accomplish this function through various methods. For example, the proto-oncogene c-cis encodes a growth factor (Reddy et al.,1988) and c-mas encodes a protein kinase (Glover and Haines, 1989).

The discovery that the protein products of some proto-oncogenes are localized to the cell nucleus suggests a possible role in the transduction of signals between the cell membrane and the nucleus. The protein encoded by the proto-oncogene c-fos has been localized to the nucleus and can bind to DNA (Morgan and Curran, 1991). C-fos was first discovered to be the transforming sequence of an osteogenic sarcoma virus, v-fos denoted the viral oncogene and c-fos is the cellular proto-oncogene from which it is derived.

Many studies have pointed to the role of c-fos as a transcriptional regulator. The first clear indication that c-fos functions in gene regulation is that it encodes a nuclear protein. Curran and Miller (1984) used immunofluorescent staining of mouse amnion cells, which have the highest level of c-fos messenger RNA,

to show localization of the protein to the cell nucleus. Further experimentation by Sambucetti and Curran (1986) showed that the fos protein is associated with chromatin and can bind DNA cellulose in vitro. A third indication of the activity of c-fos as a transcriptional regulator lies in research with platelet-derived growth factor (PDGF). Kruijer et al. (1984) discovered that the nuclear protein fos is synthesized ten minutes after exposure of quiescent mouse fibroblasts to PDGF, making this one of the earliest known transcriptional events following mitogenic stimulation. Finally, as supporting evidence for c-fos involvement in gene regulation, Distel et al. (1987) found direct participation of c-fos nucleoprotein complexes that regulate gene expression in adipocyte differentiation. Thus, there is substantial evidence to indicate a role for c-fos in gene regulation.

Basal levels of fos protein in most tissues are low but increase in specific brain areas following sensory stimulation. Induction of c-fos is now known to be closely coupled to instances which involve the excitation of nerve cells. In an attempt to further establish the role of c-fos in coupling extracellular signals to long term changes in gene expression, Morgan and Curran (1986) used agents such as veratridine and elevated potassium, which are known to depolarize neurons, to illustrate induction of c-fos by a calcium-gated channel. Cholinergic agonists have also been shown to cause c-fos activation in neuronally differentiated phaeochromocytoma (PC-12) cells (Greenberg and Ziff, 1984). The

evidence presented here shows a strong correlation between c-fos induction and nerve cell stimulation.

C-fos does not act alone in signal transduction, evidence shows that the fos gene family forms heterodimeric complexes with the jun family to bind to the AP-1 transcription factor (Cooper, 1990). Since AP-1 increases after treatment with phorbol ester, it is thought to be a component of protein kinase C mediated signal transduction pathways (Cooper, 1990). Jun and fos proteins form heterodimers through interaction of hydrophobic leucine side chains (leucine zipper) and together bind DNA to activate transcription of AP-1 target genes (Turner and Tijan, 1989).

Proto-oncogenes have also been postulated to play a role in steroid hormone action. Estrogen has been shown to increase c-fos messenger RNA in the rat uterus (Loose-Mitchell et al., 1988). Gaub et al. (1990) reported that the ovalbumin estrogen response element binds a nucleoprotein complex containing c-fos and c-jun. Schuchard et al. (1993) proposed a cascade model of steroid action in which proto-oncogenes act as rapidly responding nuclear regulators in response to hormone and can serve as markers for hormone action in tissue.

The induction of c-fos by various sensory stimuli has been reported. Rats exposed to a four hour immobilization stress showed increased fos immunoreactivity in the hypothalamic-pituitary axis (Kononen et al., 1992). C-fos has also been shown to be induced by an unconditioned stress such as foot shock and conditioned stress such as foot shock paired with a tone, in areas such as septum,

cingulate cortex, locus ceruleus, and hypothalamic paraventricular nucleus (Smith et al., 1992). Rats exposed to propionic acid as an odor stimulus also showed increased fos staining patterns in the olfactory bulb (Onoda, 1992). Since the basal level of fos is low and increases with stimulation, fos has been used as an indicator of neuronal activity.

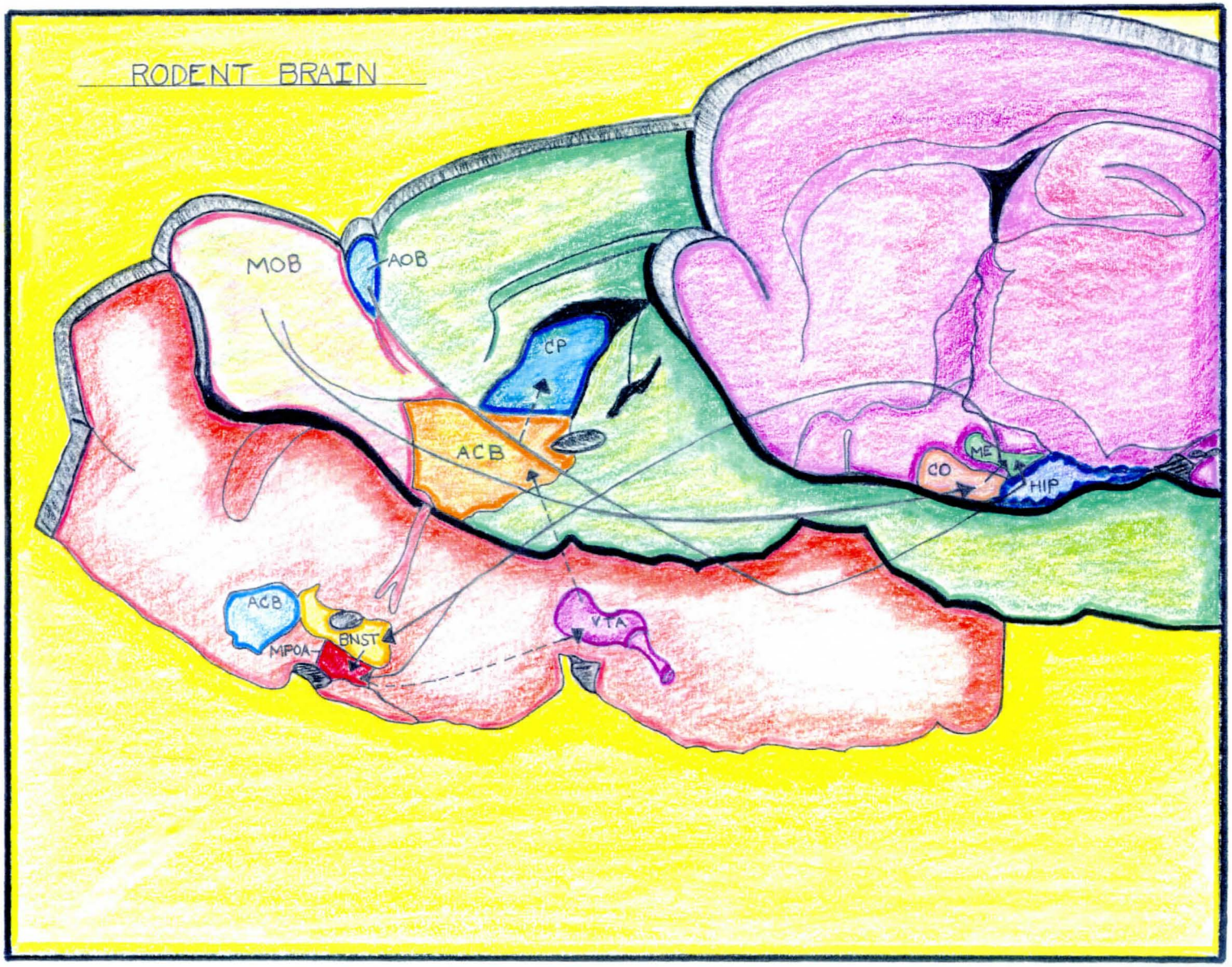
Sexual behavior has also been shown to increase fos levels in various areas of the brain. Kollack and Newman (1992) performed fos immunocytochemistry on the brains of male syrian hamsters following a single mating test. They reported increased fos expression in areas of the brain which have been shown by lesion studies to eliminate or change mating behavior; the amygdala, bed nucleus of the stria terminalis (BNST), and MPOA. In rats, the MPOA and nucleus accumbens are assumed to play a large role in the regulation of sexual activity. Robertson et al. (1991) reported increased fos in the male rat brian following copulation, in the MPOA, the nucleus accumbens, BNST, and piriform cortex. Baum and Everitt (1992) investigated the role of afferent inputs from the medial amygdala and central tegmental field and reported increased fos in the MPOA after mating in male rats. They reported that unilateral electrothermal lesions of the olfactory peduncle reduced fos in the medial amygdala, if the animals were prevented from ejaculating. This implies that olfactory inputs from the central tegmental field result in induction of c-fos in the stria terminalis and MPOA.

Although numerous studies have shown fos to increase with various types of stimulation, few studies have investigated whether c-fos is critical for certain behaviors. Baum et al. (1994) reported that homozygous mutant male mice who lack a functional c-fos gene were slower to mount and showed significantly lower mounting rates than controls. They suggested that these results may be due to an interruption in the cascade of gene transcription events that are initiated by c-fos in response to sensory stimuli. Alternately, they propose that the mutation leads to a structural change, through disturbed neural development, that is responsible for the deficit. These data certainly suggest that c-fos may prove to be a critical factor in translating the neural mechanisms that regulate sexual behavior.

The MPOA and olfactory bulbs have been shown through various experiments to have a profound effect on copulatory behavior in male rats. Since the nucleus accumbens is seen as a relay center for neural messages acquired from the motivational state of the animal to motor neurons in the basal ganglia, this region may also be involved in sexual behavior. Lesion studies have been important in contributing to our knowledge of sexual behavior but some difficulties are inherent. Results of lesion studies can be affected by the severity of the lesion, that is, how much of the brain area is destroyed. The use of fos as a marker of neuronal activity has the advantage of specificity and the advantage that the entire brain area is left intact. The purpose of the present study was to determine the effects of exposure of sexually

experienced male rats to female pheromones using fos immunostaining as an indicator of neuronal activity. Furthermore, the effects of castration on pheromone stimulated fos-staining were investigated.

Figure 1. Three parasagittal cross sections of the rodent brain demonstrating neural pathways between brain areas. Abbreviations: AOB=Accessory olfactory bulbs; ACB=Nucleus accumbens; BNST=Bed nucleus of the stria terminalis; CO=Cortical amygdala; CP=Caudate putamen; HIP=Hippocampus; ME=Medial amygdala; MPOA=Medial preoptic area; VTA=Ventral tegmental area (Figure adapted from Sachs and Meisel, 1988).


RODENT BRAIN
MOB
AOB
CP
ACB
ME
CO
HIP
ACB
MPOA
BNST
VTA

# CHAPTER II

## MATERIALS AND METHODS

### ANIMALS

Sexually mature Long-Evans rats age 3-6 months were housed separately in groups of three to four with a reversed 12h light: 12h dark cycle, with lights off at 1000h. Food and water were always available. Six female rats were bilaterally ovariectomized and used as controls in the experiments. Another six females were brought into estrous by injection of estradiol benzoate (50μg/.1ml) 48 hours prior to testing and injection of progesterone (500μg/.1ml) 5-7 hours before testing. All males were pretested for sexual behavior with a receptive female until 2 ejaculations were experienced.

### EXPERIMENTAL DESIGN

EXPERIMENT 1. The effects of exposure of intact sexually experienced males to the bedding of estrous females on neuronal expression of fos was assessed. Twelve male rats were randomly divided into control and experimental groups, with six in each. Each male was placed into a clean glass observation chamber with bedding samples gathered from ovariectomized females (control) or bedding samples gathered from estrous females. Both groups were

exposed to the bedding for 2 hours. Following exposure, the rats were deeply anesthetized, perfused, and brains were prepared for immunocytochemical analysis for fos as described below.

EXPERIMENT 2. The effect of castration on the males' neuronal expression of fos in response to bedding from estrous or ovariectomized females was assessed. Twelve sexually experienced male rats were castrated and randomly divided into control and experimental groups, six in each. Males were placed separately into clean glass observation chambers with bedding samples collected from ovariectomized females (control) or from estrous females. As in experiment one, both groups were exposed to the bedding samples for 2 hours. Following exposure, the rats were deeply anesthetized, perfused, and their brains were prepared for immunocytochemical analysis for fos as described below.

IMMUNOCYTOCHEMISTRY

Rats were deeply anesthetized with Ketamine (50mg/kg) and Rompun (4mg/kg) and perfused intracardially with 30 ml of physiological saline followed by 100 ml of Modified Zamboni's Fixative (150ml saturated picric acid and 20g paraformaldehyde brought to 1L with phosphate buffer). The olfactory bulbs were removed, placed in 10% formalin, and refrigerated overnight. Brains were trimmed, placed in Modified Zamboni's Fixative or 10% formalin, and refrigerated overnight. Brains fixed in Zamboni's were sectioned on a vibratome at 100$\mu$m thickness. Brains fixed in formalin and olfactory bulbs were sectioned on a freezing microtome

at 75μm thickness. Ten consecutive sections were saved at the level of MPOA and nucleus accumbens, and ten consecutive sections of olfactory bulbs were saved.

Sections were washed twice with tris buffered saline (TBS) (.1% Bovine serum albumin, .1% Triton-X. .01% Merthiolate) followed by incubation with 1.0% hydrogen peroxide in TBS to block endogenous peroxidase activity. Sections were then incubated for 48 hours with a primary polyclonal antibody (c-fos-Ab-2;Oncogene Science) developed in rabbit. The antibody (100μg/ml)was diluted 1:500 with TBS. To insure specificity of the antibody, several sections were incubated with a mixture of excess fos antigen (100μg/1ml) (1:50 dilution) and fos antibody (1:500 dilution). These sections were later determined to lack fos staining, thus assuring specificity of the antibody. Following incubation with primary antibody, the sections were washed with TBS and incubated 1 hour with a biotinylated secondary antibody. The sections were then washed with TBS followed by a 1 hour incubation with the Vectastain ABC reagent. The sections were rinsed with TBS. The reaction was visualized using diaminobenzidine (3,3-diaminobenzidine tetrahydrochloride; Sigma) as a chromogen. Sections were then floated onto albuminized glass slides, counterstained with methylene blue, dehydrated through an ethanol series to xylene, and coverslipped using permount as the mounting medium.

To quantify the number of fos stained nuclei, sections were examined microscopically and dark brown stained nuclei were counted

in three consecutive sections of each brain area, Nucleus accumbens, MPOA, corpus striatum, diagonal band of broca, and olfactory bulbs.

STATISTICAL ANALYSIS

The data were analyzed for differences in number of fos stained nuclei by two-way analyses of variance (ANOVA). Where significant treatment effects were detected by ANOVA they were further probed by the Newman-Keuls test. Significance was inferred when $p \leq 0.05$ in two tailed probability tests.

# CHAPTER III

## RESULTS

Fos staining was seen in the nucleus accumbens, MPOA, corpus striatum, diagonal band of broca, and olfactory bulbs of all animals with generally lower numbers in castrated animals. The data were examined according to brain region in intact and castrated rats.

### Medial Preoptic Area

The number of fos stained nuclei in the MPOA of intact male rats exposed to bedding from estrous females was significantly greater than controls (figure 2, $p \leq .005$). Castrated rats exposed to estrous bedding showed no increase in fos staining compared to controls (figure 2).

### Nucleus Accumbens

The nucleus accumbens showed a dramatic increase in fos stained nuclei in intact males exposed to bedding from estrous females compared to controls (figure 3, $p \leq .001$). Castrated males exposed to estrous bedding showed no increase in fos stained nuclei over controls (figure 3).

Corpus Striatum

Intact males exposed to bedding from estrous females also demonstrated a slight, but significant increase over controls in the corpus striatum($p \leq .05$), while no such increase was observed in castrated males exposed to bedding from ovariectomized females (figure 4).

Olfactory Bulbs

The olfactory bulb sections of all animals showed intense staining in all sections. No significant differences were seen between groups (figure 5).

Diagonal Band of Broca

The DBB was not initially included as an area of examination but upon examination of sections, staining was noted in this area. The DBB showed a significant increase in fos stained nuclei for intact males exposed to estrous bedding as compared to controls (figure 6, $p \leq .005$). In contrast to results from other brain areas, castrated males exposed to estrous bedding also showed a significant increase in fos stained nuclei over controls (figure 6, $p \leq .025$).

Figure 2: Number of fos-stained nuclei in the MPOA of intact and castrated male rats. Abbreviations in this and subsequent figures: C=Controls exposed to ovariectomized bedding; E=Experimental males exposed to estrous bedding.

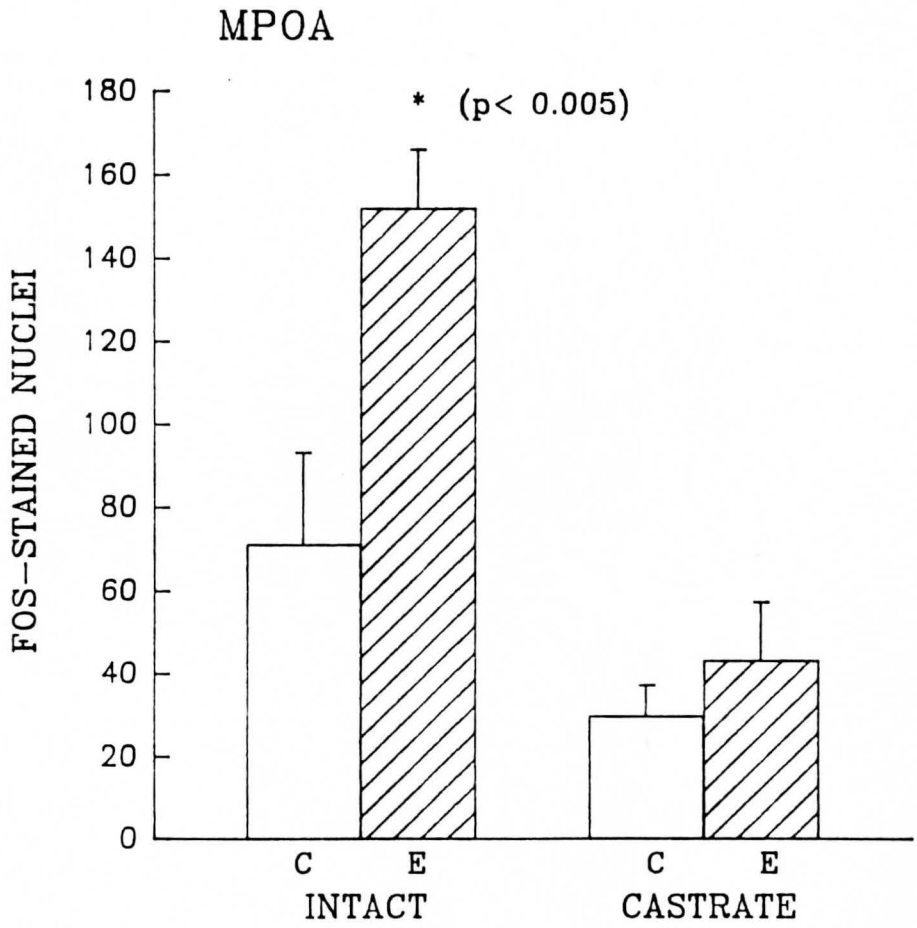

MPOA
* (p< 0.005)
FOS-STAINED NUCLEI
180
160
140
120
100
80
60
40
20
0
C
E
C
E
INTACT
CASTRATE

Figure 3: Number of fos-stained nuclei in the nucleus accumbens (Nac) of intact and castrated rats.

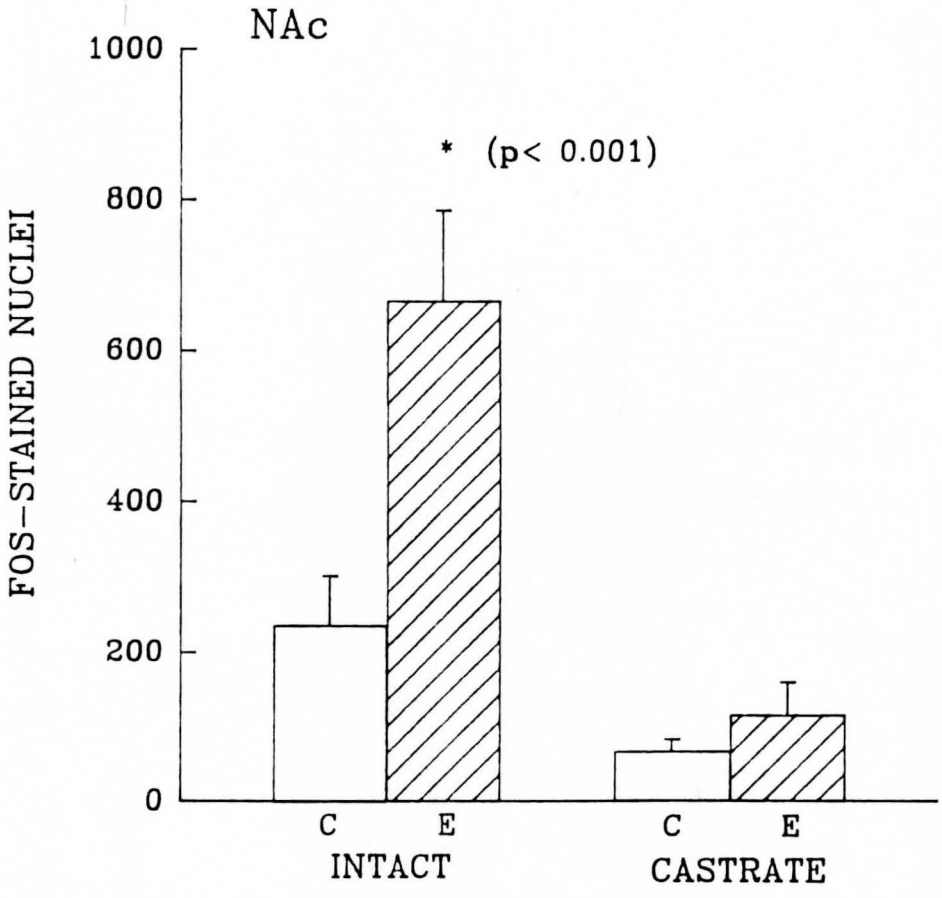

NAc
1000
800
600
400
200
0
FOS-STAINED NUCLEI
* (p< 0.001)
C
E
INTACT
C
E
CASTRATE

Figure 4: Number of fos-stained nuclei in the corpus striatum (CPU) of intact and castrated rats.

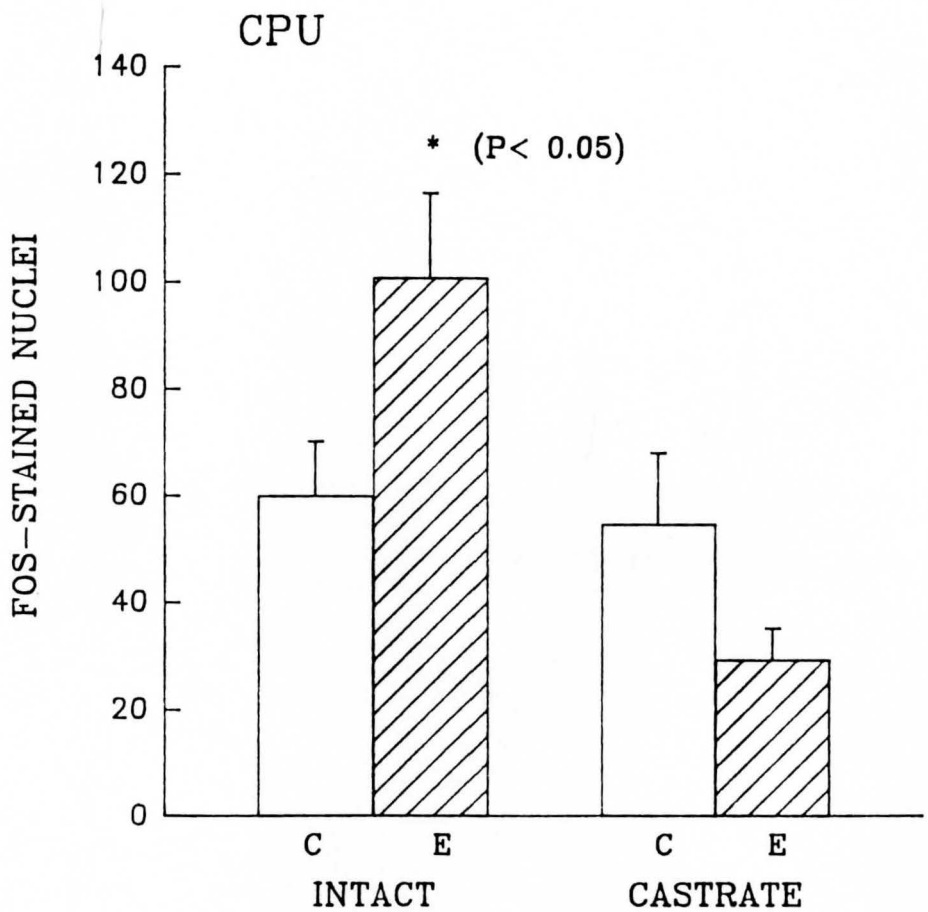

CPU
140
120
100
80
60
40
20
0
FOS-STAINED NUCLEI
* (P< 0.05)
C
E
C
E
INTACT
CASTRATE

Figure 5: Number of fos-stained nuclei in the olfactory bulbs (OB) of intact and castrated rats.

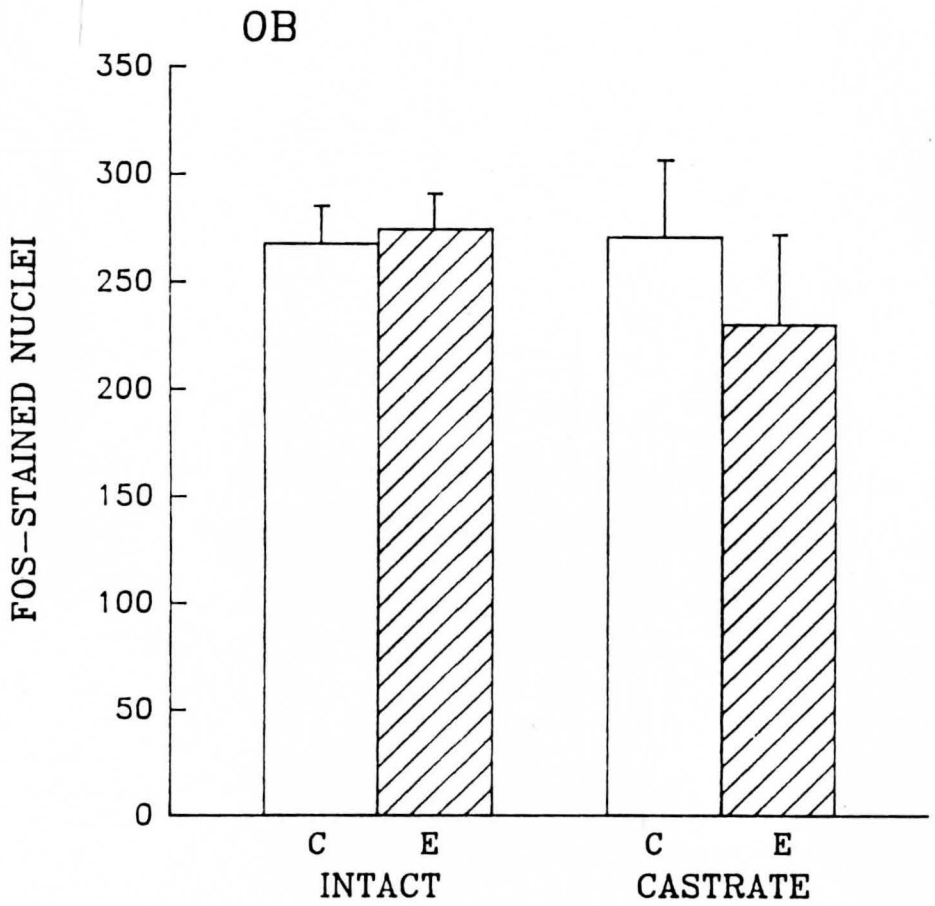
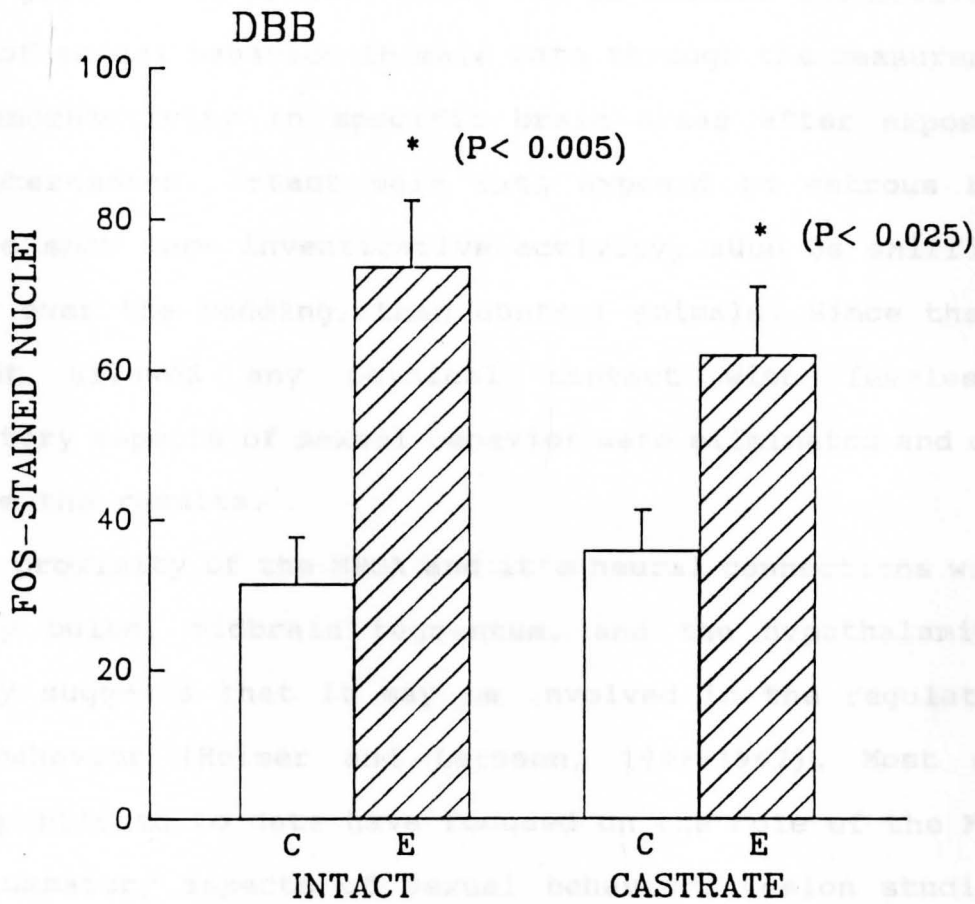

OB
FOS-STAINED NUCLEI
350
300
250
200
150
100
50
0
C
E
C
E
INTACT
CASTRATE

Figure 6: Number of fos-stained nuclei in the diagonal band of broca (DBB) of intact and castrated rats.


DBB
FOS-STAINED NUCLEI
100
80
60
40
20
0
* (P< 0.005)
* (P< 0.025)
C
E
C
E
INTACT
CASTRATE

# CHAPTER IV

## DISCUSSION

The goal of the present study was to examine the motivational aspects of sexual behavior in male rats through the measurement of fos immunoreactivity in specific brain areas after exposure to female pheromones. Intact male rats exposed to estrous bedding tended to show more investigative activity, such as sniffing and climbing over the bedding, than control animals. Since the males were not allowed any physical contact with females, the consummatory aspects of sexual behavior were eliminated and did not influence the results.

The proximity of the MPOA and it's neural connections with the olfactory bulbs, midbrain tegmentum, and the hypothalamic area certainly suggests that it may be involved in the regulation of sexual behavior (Heimer and Larsson, 1966/1967). Most of the studies published to date have focused on the role of the MPOA in the consummatory aspects of sexual behavior. Lesion studies and electrical stimulation of the MPOA have shown that this area has a function in the regulation of sexual behavior in the male rat. In addition, other studies have suggested that the MPOA, part of the limbic system, may play a role in establishing the motivational state of the animal. A study by Chen and Bliss (1974) reported that incomplete ablation of the MPOA increased mount, intromission, and ejaculation latencies while the post ejaculatory-refractory interval was not changed. They suggested that the MPOA is involved

in a sexual arousal mechanism. Baum et al. (1992) reported no increase in fos in the MPOA of male rats exposed to an estrous female enclosed in a plexiglass box but noted increased fos with increasing physical contact with females. In the present study, males were allowed direct physical contact with the bedding, thus enhancing olfactory stimulation. We observed a significant increase in fos stained nuclei in the MPOA of intact male rats that were exposed to bedding gathered from estrous females. Our results support the hypothesis that the MPOA may be involved in establishing the motivational state of the animal.

The importance of testosterone in male sexual behavior is well documented. Sar and Stumpf (1973) showed by autoradiographic methods that testosterone binds to cells of the MPOA. Davidson (1966) reported that implantation of crystalline testosterone propionate into the MPOA was effective in restoring sexual behavior in castrated male rats. In our study, castration prevented the increase in neural activity in the MPOA in response to olfactory cues from estrous females as measured by fos immunoreactivity. Our results support a role for testosterone in mediating the motivational aspects of sexual behavior by acting in the MPOA of the male rat.

The nucleus accumbens is thought to function in the translation of motivation into action. Limbic structures of the forebrain have neural connections with the ventral tegmental area which projects dopaminergic neurons into the nucleus accumbens. Dopamine levels in the nucleus accumbens are increased by

copulatory behavior (Damsma et al.,1992) as well as by various stimuli associated with sexual behavior (Pfaus et al.,1990). Levels of dopamine in the nucleus accumbens increase with exposure of male rats to the bedding of estrous females, suggesting that the nucleus accumbens may be involved in pairing stimuli with reward processes (Louilat et al.,1986). Sexual behavior also increases fos in the nucleus accumbens of male rats (Robertson et al.,1991). In their study, the males were allowed to copulate and fos levels were not examined before copulation. In the present study, the males were not permitted any contact with females. Further experimentation is needed to determine if the increase in fos in the nucleus accumbens is incremental with increased physical contact with the female and with ejaculation. The increase in fos staining in the nucleus accumbens we observed further supports a role for the nucleus accumbens on the processing of motivational cues from the environment.

Mitchell and Stewart (1989) reported a decrease in dopamine in the nucleus accumbens of castrated male rats that was prevented by treatment with testosterone. They suggested that testosterone may act on the mesolimbic dopamine system to influence sexual arousal in the male rat. In our experiment, castration prevented activation of neurons in the nucleus accumbens after exposure to bedding from estrous females. These results support a role for testosterone in mediating the response of the nucleus accumbens neurons to sexually relevant olfactory cues received from the environment.

Dopamine in the corpus striatum may mediate the motor activity of copulation. Copulatory activity increases dopamine levels in the corpus striatum of male rats (Damsma et al.,1992). The increased fos staining seen in the corpus striatum of intact male rats exposed to estrous bedding correlates with the dopamine increase. In contrast, Robertson et al. (1991) reported no increase in fos staining in the corpus striatum of copulating male rats. Further experimentation is necessary to determine whether the increase in fos in experimental rats is due to the increased motor activity observed when males were exposed to estrous bedding. The increase in fos is consistent with the function of the striatum in the final integration of sensory data.

Evidence suggests that the olfactory bulbs are involved in the regulation of sexual behavior in the male rat. Early studies by Heimer and Larsson (1967) showed that olfactory lesions of experienced male rats increased latencies, decreased ejaculations, and increased the probability of unresponsiveness to the female. Theories arose suggesting that the olfactory bulbs have a role in modulating the limbic system in addition to their well known sensory role (Cain, 1974). It has also been suggested that the olfactory bulbs are involved in a central arousal mechanism. For example, non-ejaculating bulbectomized male rats subjected to tail pinch prior to exposure to an estrous female can be stimulated to copulate (Wang and Hull, 1980). Tail pinch, which is considered to be an arousing stimulus, may act to stimulate an arousal mechanism or it may focus the rats' attention on cues that are relevant to

sexual behavior. We observed intense staining of the olfactory bulbs in all control and experimental groups. All groups were exposed to general room air and the only variable between groups was exposure to bedding from either estrous or ovariectomized females. Therefore, it is conceivable that the intense staining in the olfactory bulbs from control and experimental males was due to the accumulation of all olfactory inputs. Additional experiments would have to be designed to investigate the effects of selective olfactory inputs on c-fos activity in the olfactory bulbs.

Although little information is available concerning a role for the DBB in the mediation of sexual activity, this brain region has been reported to function as a relay between areas of the limbic system and the olfactory cortex and may participate in associative memory storage (Roman et al.,1993). Our results demonstrated an increase in fos staining in the DBB of both intact and castrated males exposed to estrous bedding. These results suggest that this mechanism may be independent of testosterone since castrated rats responded to estrous bedding in the same manner as intact rats. Further experiments are needed to investigate the role of the DBB in regulating sexual behavior.

The MPOA and nucleus accumbens may be involved in processing sexually relevant cues from the environment to establish the motivational state of the animal. The increase in fos staining seen in these two brain areas in intact males after exposure to pheromones from estrous females supports this theory. Because castration prevented the increase in fos staining in the MPOA and

nucleus accumbens in response to female pheromones, our results support a role for testosterone in mediating the responses of the MPOA and nucleus accumbens to sexually relevant cues.

REFERENCES

Baum M.J., Brown J.J.G., Kica E., Rubin B.S., Johnson R.S., and Papaionnou V.E. (1994) Effect of a Null Mutation of the c-fos Proto-oncogene on Sexual Behavior of Male Mice. Biology of Reproduction. 50, 1040-1048.

Baum M.J. and Everitt B.J. (1992) Increased Expression of c-fos in the Medial Preoptic Area After Mating in Male Rats: Role of Afferent Inputs from the Medial Amygdala and Midbrain Central Tegmental Field. Neuroscience. 50 (3), 627-646.

Bermant G. and Taylor L. (1969) Interactive Effects of Experience and Olfactory Bulb Lesions in Male Rat Copulation. Physiology and Behavior. 4, 13-17.

Brackett N.L. and Edwards D.A. (1984) Medial Preoptic Connections with the Midbrain Tegmentum are Essential for Male Sexual Behavior. Physiology and Behavior. 32, 79-84.

Cain D.P. (1974) The Role of the Olfactory Bulb in Limbic Mechanisms. Psychological Bulletin. 61 (10), 654-671.

Chen J.J. and Bliss D.K. (1974) Effects of Sequential Preoptic and Mammillary Lesions on Male Rat Sexual Behavior. Journal of Comparative and Psychological Psychology. 87 (5), 841-847.

Cooper G.M. (1990) Oncogenes. Jones and Bartlett, Boston.

Curran T., Miller D.A., and Verma I.M. (1984) Viral and Cellular fos Proteins: A Comparative Analysis. Cell. 36, 259-268.

Damsma G., Pfaus J.G., Wenkstern D., Phillips A.G., and Fibiger H.C. (1992) Sexual Behavior Increases Central Dopamine Transmission in the Nucleus Accumbens and Striatum of Male Rats: Comparison with Novelty and Locomotion. Behavioral Neuroscience. 106 (1), 181-191.

Davidson J. (1966) Activation of the Male Rats Sexual Behavior by Intracerebral Implantation of Androgen. Endocrinology. 79, 783.

deGroot J. (1991) Correlative Neuroanatomy. Appleton and Lange, Norwalk, Connecticut.

Distel R.J., Ro H., Rosen B.S., Groves D.L., and Spiegelman B.M. (1987) Nucleoprotein Complexes that Regulate Gene Expression in Adipocyte Differentiation: Direct Participation of C-fos. Cell. 49, 835-844.

Edwards D.A. and Einhorn L.C. (1986) Preoptic and Midbrain Control of Sexual Motivation. Physiology and Behavior. 37, 329-335.

Gaub M., Bellard M., Scheuer I., Chambon P., and Sassone-Corsi P. (1990) Activation of the Ovalbumin Gene by the Estrogen Receptor Involves the Fos-Jun Complex. Cell. 63, 1267-1276.

Glover D.M. and Haines B.D. (1989) Oncogenes. Oxford University Press.

Greenburg M.E. and Ziff E.B. (1984) Stimulation of 3T3 Cells Induces Transcription of the c-fos Proto-Oncogene. Nature. 311, 422-437.

Heimer L. and Larsson K. (1966/67) Impairment of Mating Behavior in Male Rats Following Lesions in the Preoptic-Anterior Hypothalamic Continuum. Brain Research. 3, 248-263.

Heimer L. and Larsson K. (1967) Mating Behavior of Male Rats after Olfactory Bulb Lesions. Physiology and Behavior 2, 207-209.

Hjorth-Simonsen A. (1971) Hippocampal Efferents to the Ipsilateral Entorhinal Area: An Experimental Study in the Rat. Journal of Comparative Neurology. 142, 417-483

Hlinak Z. (1984) Influence of Repeated Exposure of Male Rats to Oestrus Odour on Their Subsequent Sexual Behavior. Activatas Nervosa Superior. 26 (2), 112-117.

Kollack S.S. and Newman S.W. (1992) Mating Behavior Induces Selective Expression of Fos Protein within the Chemosensory Pathways of the Male Syrian Hamster Brain. Neuroscience Letters. 143, 223-228.

Kononen J., Honkaniemi J., Alho H., Koistinaho J., Iadarola M., and Pelto-Huikko M. (1992) Fos-Like Immunoreactivity in the Rat Hypothalamic-Pituitary Axis After Immobilization Stress. Endocrinology. 130 (2), 3041-3047.

Kruijer W., Cooper J.A., Hunter Y., and Verma I.M. (1984) Platelet Derived Growth Factor Induces Rapid But Transient Expression of the c-fos Gene and Protein. Nature. 312, 711-716.

Larsson K. (1969) Failure of Gonadal and Gonadotropic Hormones to Compensate for an Impaired Sexual Function in Anosmic Male Rats. Physiology and Behavior. 4, 733-737.

Larsson K. (1975) Sexual Impairment of Inexperienced Male Rats Following Pre- and Postpubertal Olfactory Bulbectomy. Physiology and Behavior. 14, 195-199.

Loose-Mitchell D.S., Chiapetta C., and Stances G.M. (1988) Estrogen Regulation of c-fos Messenger Ribonucleic Acid. Molecular Endocrinology. 2, 946-951.

Louilot A., Lemoal M., and Simon H. (1986) Differential Reactivity of Dopaminergic Neurons in the Nucleus Accumbens in Response to Different Behavioral Situations. An In Vivo Voltametric Study in Free Moving Rats. Brain Research. 397, 395-400.

Louilot A., Gonzalez-Mora J.L., Guadalupe T., and Mas M. (1991) Sex Related Olfactory Stimuli Induce Selective Increase in Dopamine Release in the Nucleus Accumbens of Male Rats. A Voltametric Study. Brain Research. 533, 313-317.

Lumia A.R., Teicher M.H., Salchi F., Ayers E., and Possidente B. (1992) Olfactory Bulbectomy as a Model for Agitated Hyposerotonergic Depression. Brain Research. 587, 181-185.

Luskin M.B. and Price J.L. (1982) The Topographic Organization of Associational Fibers of the Olfactory System of the Rat Including Centrifugal Fibers to the Olfactory Bulb. Journal of Comparative Neurology. 216, 264-291.

Malmnas C.F. (1976) The Significance of Dopamine, versus Other Catecholamines, for L-Dopa Induced Facilitation of Sexual Behavior in the Castrated Male Rat. Physiology and Behavior. 4, 521-526.

Malsbury C. (1971) Facilitation of Male Rat Copulatory Behavior by Electrical Stimulation of the Medial Preoptic Area. Physiology and Behavior. 7, 797-805.

Mas M., Gonzalez-Mora J.L., Louilot A., Sole C., and Guadalupe T. (1990) Increased Dopamine Release in the Nucleus Accumbens of Copulating Male Rats as evidenced by In Vivo Voltametry. Neuroscience Letters. 110, 303-308.

McCullough L.D. and Salamone J. (1992) Involvement of Nucleus Accumbens Dopamine in the Motor Activity Induced by Periodic Food Presentation: A Microdialysis and Behavioral Study. Brain Research. 592, 29-36.

Meisel R.L., Lumia A.R., and Sachs B.D. (1980) Effects of Olfactory Bulb Removal and Flank Shock on Copulation in Male Rats. Physiology and Behavior. 25, 383-387.

Mitchell J.B. and Stewart J. (1988) Effects of Castration, Steroid Replacement, and Sexual Behaviors in the Male Rat. Brain Research. 491, 116-127.

Mitchell J.B. and Gratton A. (1991) Opioid Modulation and Sensitization of Dopamine Release Elicited by Sexually Relevant Stimuli: A High Speed Chronoamperometric Study in Freely Behaving Male Rats. Brain Research. 551, 20-27.

Schuchard M., Landers J.P., Sandhu N.P., and Spelsberg T.C. (1993) Steroid Hormone Regulation of Nuclear Proto-oncogenes. Endocrine Reviews. 14, 659-669.

Smith M.A., Banerjee S., Gold P.W., and Glowa J. (1992) Induction of C-Fos mRNA In Rat Brain by Conditioned and Unconditioned Stressors. Brain Research. 578, 135-141.

Tagliamonte A., Fratta W., Del Fiacco M., and Gessa G.L. (1974) Possible Stimulatory Role of Brain Dopamine in the Copulatory Behavior of Male Rats. Pharmacology Biochemistry and Behavior. 2, 257-260.

Turner R. and Tijan R. (1989) Leucine Repeats and an Adjacent DNA Binding Domain Mediate the Formation of Functional C-Fos-C-Jun Heterodimers. Science. 243, 1689-1692.

van De Poll N.E. and van Dis H. (1977) The Effect of Medial Preoptic-Anterior Hypothalamic Lesions on Bisexual Behavior of the Male Rat. Brian Research Bulletin. 4, 505-511.

Vandenberg, J.G. Pheromones and Mammalian Reproduction. In: The Physiology of Reproduction. Ed. Knobil, E. et al. Volume 2 New York. Raven Press. 1988:1679-1696.

van Dis H. and Larsson K. (1971) Induction of Sexual Arousal in the Castrated Male Rat by Intracranial Stimulation. 6, 85-86.

Wang L. and Hull E.M. (1980) Tail Pinch Induces Sexual Behavior on Olfactory Bulbectomized Male Rats. Physiology and Behavior. 24, 211-215.

West H.K., Clancy A.N., and Michael R.P. (1992) Enhanced Responses of Nucleus Accumbens Neurons in Male Rats to Novel Odors Associated with Sexually Receptive Females. Brain Research. 585, 49-55.